

Supplementary figure legends

Fig. S1. Representative H&E staining of HCC and paired peri-tumor tissue.

Fig. S2. Kaplan-Meier survival analysis was performed to investigate the impact of the methylation level of MCM2 enhancer (cg08889930 site) on overall survival of patients from TCGA cohort (n = 374). *p* is based on log-rank test.

Fig. S3. Validation of the methylation pattern of the indicated CpG islands. (A) Results from pyrosequencing showing that the methylated percentage of CpG sites of MCM2 enhancer region (cg0889930 site) was significantly lower in HCC samples than paired nontumor samples. (B) Pyrosequencing results showing that the methylated percentage of CpG sites of CG0350244 was similar between HCC and than paired nontumor samples. (C) Pyrosequencing results showing that the methylated percentage of CpG sites of CG03502446 was significantly higher in HCC samples than in paired nontumor samples. (D) Box plot showing the mean methylated percentage of CpG sites at NUP37 promoter region was significantly higher in HCC as compared with paired nontumor samples.

Fig. S1

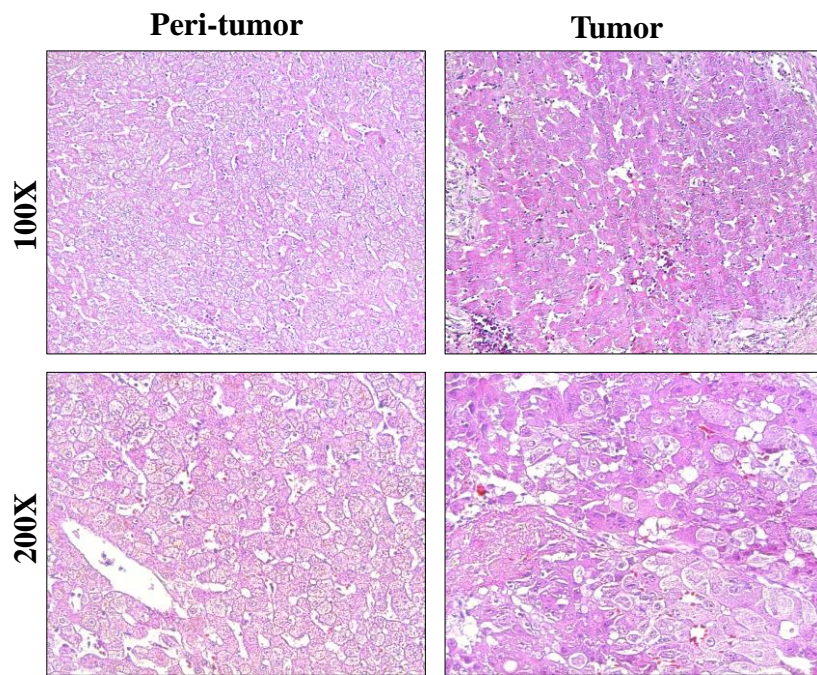


Fig. S2

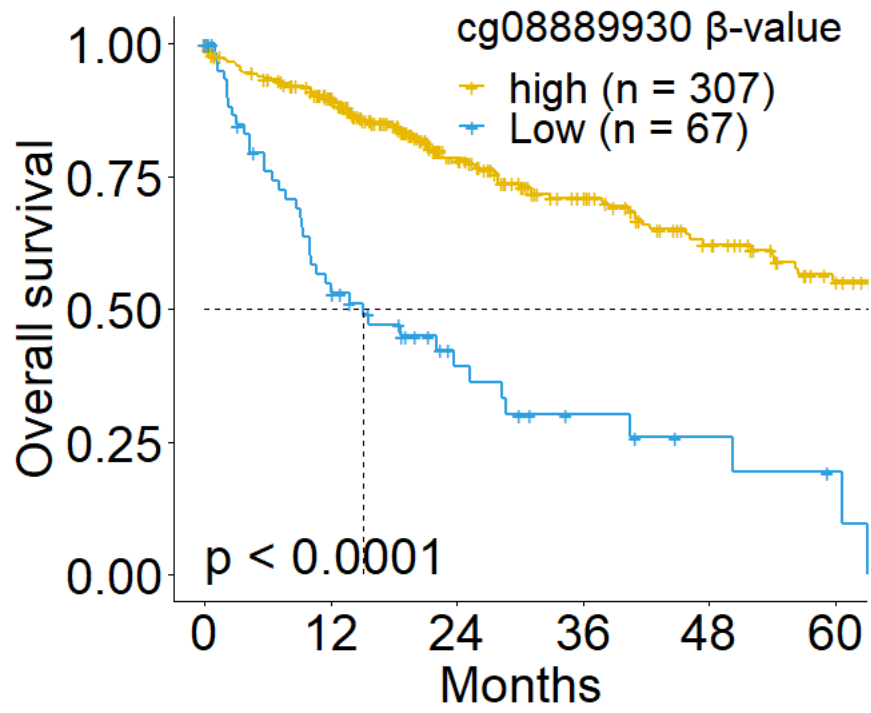
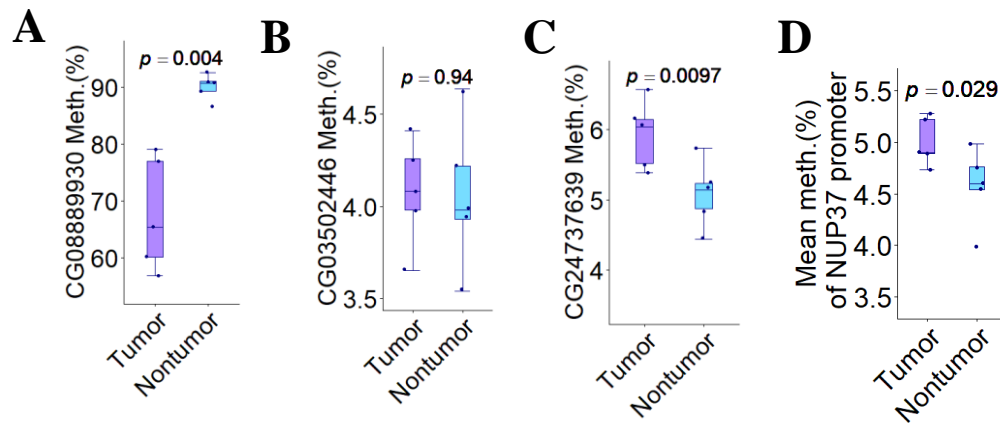


Fig. S3



Supplementary Table 1. Clinicopathological Features of HCC Patients (n = 300)

Age, median (range), year	61 (18-88)
Gender (F: M), n (%)	39 (13.0): 261 (87.0)
Histological grade, n (%)	
Low/Low-moderate	67 (22.3)
Moderate/Moderate-well	207 (69.0)
Well	26 (8.7)
Tumor size, mean (range), cm	4.8 (1.0-18.0)
AFP, mean (range), ug/L	2434 (1-200000)
ALT, mean (range), U/L	46 (6-702)
AST, mean (range), U/L	56 (11-1597)
T-stage, n (%)	
T1a	63 (21.0)
T1b	105 (35.0)
T2	90 (30.0)
T3	11 (3.7)
T4	31(10.3)
N-stage, n (%)	
N0	291 (97.0)
N1	2 (2.0)
Nx	1(1.0)
M-stage, n (%)	
M0	289 (96.3)
M1	11 (3.7)
TNM-stage, n (%)	
IA/IB	63 (21.0)/102 (34.0)
II	86 (28.7)
IIIA/IIIB	12 (4.0)/24 (8.0)
IVA/IVB	5 (1.7)/8 (2.7)
Hypertension (yes: no), n (%)	76 (25.3): 224 (74.7)
Diabetes (yes: no), n (%)	28 (9.3): 272 (90.7)
Liver cirrhosis (yes: no), n (%)	31 (10.3): 269 (89.7)
Fatty liver (yes: no), n (%)	32 (10.7): 268 (89.3)
Hepatitis B virus (yes: no), n (%)	221 (73.7): 79 (26.3)

HCC, hepatocellular carcinoma; F, female; M, male; AFP, α -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase;

Supplementary Table 2. Primer sequences and location of CpG sites tested using bisulfite pyrosequencing

Sequence name	primer	Sequence (5' → 3')	Sequence to Analyze	Strand	CpG site location (hg19)
CG08889930	Forward	/5'Biotin/TTTGTTTTGGTGGTTTTTTTATGTTAT	RCAAAATATT ATAATAAAAA TAACAT	+	chr3:127317798 (CG1)
	Reverse	CTCCCCTTAAACATTAAACCTATTCA			
	Sequencing	ATTCAACACACACAATAC			
CG03502446 & CG24737639	Forward	GGAAGGAAGAATTTTTGGGAATAG	GGTTGGGYGG TYGGTAGAAA AGTATTT	+	chr12:102513777 (CG2)
	Reverse	/5'Biotin/CCAAAAAACCACCTACCTCTAA			chr12:102513781 (CG3)
	Sequencing	TGGGAATAGAGTGGTAA			

/5Biosg/ = 5' biotin added